

In re Appln. of Feussner et al.  
Application No. Unassigned (U.S. National Phase of PCT/EP00/06539)

composition of the hydroxy fatty acids was analyzed by chiral-phase HPLC on a Chiralcel OD column (Daicel Chem. Industries, distributed by Baker Chem., Deventer, Netherlands; 250 x 4.6 mm, 5µm particle size) with a solvent system of hexane/2-propanol/acetic acid (100/5/0.1, v/v/v) at a flow rate of 1 ml/min. (Cf. Feussner, I., Balkenhohl, T.J., Porzel, A., Kühn, H. & Wasternack, C. (1997) J. Biol. Chem. 272, 21635-21641).

*Amendments to the paragraph beginning at page 9, line 10:*

The starting cDNA and the mutagenesis kit were as described above. For analysis of the mutation further conservative base exchanges were carried out for producing a new restriction cleavage site for BstBL. The following primers were used for producing the mutation V576F: GCT GGT GGG GTT CTT GAG AGT ACA TTC TTT CCT TCG AAA TTT GCC ATG GAA ATG TCA GCT G (coding strand) [SEQ ID NO:1] and CAG CGT ACA TTT CCA TGG CAA ATT TCG AAG GAA AGA ATG TAC TCT CAA GAA CCC CAC CAG C (complementary strand) [SEQ ID NO:2]. Furthermore, the mutant was sequenced and 5 different bacterial colonies were expressed and used for enzymatic studies. The expression of pET-LOX1 was carried out as described above. The further preparation was carried out as already indicated above. Analysis of the produced fatty acid derivative (containing a hydroperoxy group at position 11) was carried out as indicated above. The result of the SP-HPLC analysis for converting arachidonic acid with V576F is shown in Fig. 2. The following Table 2 shows a comparison of the specificity of the wild type (wtLOX) with the mutant (LOXV<sub>576F</sub>).

*Amendments to existing claims:*

[1. A method of enhancing the specificity of a plant lipoxygenase for position 11 of arachidonic acid, comprising the step of

- exchanging at least one amino acid in a wild type lipoxygenase, characterized in that the exchange takes place at position 576 of potato tuber lipoxygenase or at a corresponding position in a lipoxygenase of another plant species.]

[2. The method according to claim 2, characterized in that the exchange at position 576 leads to the presence of a Phe residue in the mutant.]

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[3. The method according to one of claims 1 or 2, characterized in that the amino acid exchange is effected by directed mutagenesis.]

[4. Lipxygenase obtainable by a method according to any one of claims 1 to 3.]

[5. Nucleic acid coding for a lipxygenase according to claim 4.]

[6. Vector containing a nucleic acid according to claim 5.]

[7. Cell containing a nucleic acid according to claim 5 and/or a vector according to claim 6.]

[8. Plant or plant part comprising a host cell according to claim 7.]

[9. A method for producing 11-perhydroxy arachidonic acid or the reduced 11-hydroxy derivative, comprising the step of

- converting arachidonic acid with a lipxygenase according to claim 6 and, optionally, reducing the perhydroxy compound obtained to hydroxy compound.]

[10. Use of a lipxygenase according to claim 4 for producing 11-perhydroxy arachidonic acid and/or 11-hydroxy arachidonic acid.]

[11. Arachidonic acid derivative containing a hydroxy group at position 11.]

12. A method of enhancing the specificity of a plant lipxygenase for position 11 of arachidonic acid comprising changing at least one amino acid in a wild type plant lipxygenase, characterized in that the change takes place at position 576 of potato tuber lipxygenase or at a corresponding position in a lipxygenase of another plant species, whereupon the specificity of the plant lipxygenase for position 11 of arachidonic acid is enhanced.

13. The method according to claim 12, characterized in that the change at position 576 results in the presence of a Phe residue at position 576.

14. The method according to claim 12, characterized in that the amino acid change is effected by directed mutagenesis.

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15. The method according to claim 13, characterized in that the amino acid change is effected by directed mutagenesis.

16. An isolated or purified lipxygenase obtainable by the method of claim 12.

17. An isolated or purified lipxygenase obtainable by the method of claim 13.

18. An isolated or purified nucleic acid encoding the lipxygenase of claim 16.

19. An isolated or purified nucleic acid encoding the lipxygenase of claim 17.

20. An isolated or purified vector comprising the nucleic acid of claim 18.

21. An isolated or purified vector comprising the nucleic acid of claim 19.

22. A cell comprising the nucleic acid of claim 18 and/or a vector comprising said nucleic acid.

23. A cell comprising the nucleic acid of claim 19 and/or a vector comprising said nucleic acid.

24. A plant or a plant part comprising the cell of claim 22.

25. A plant or a plant part comprising the cell of claim 23.

26. A method for producing 11-perhydroxy arachidonic acid or the reduced 11-hydroxy derivative thereof comprising incubating arachidonic acid with the lipxygenase of claim 16 under appropriate conditions, whereupon 11-perhydroxy arachidonic acid is obtained, and, optionally, reducing the 11-perhydroxy arachidonic acid, whereupon the reduced 11-hydroxy derivative thereof is obtained.

27. A method for producing 11-perhydroxy arachidonic acid or the reduced 11-hydroxy derivative thereof comprising incubating arachidonic acid with the lipxygenase of claim 17 under appropriate conditions, whereupon 11-perhydroxy arachidonic acid is obtained,